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Renal Function Tests

Kidney is a highly specialized organ that performs following functions:
• **Maintenance of extracellular fluid volume and composition:** Kidney regulates water and electrolyte balance, acid-base balance, and fluid osmotic pressure.
• **Excretion of metabolic waste products** (blood urea, creatinine, uric acid) and drugs, but retention of essential substances (like glucose and amino acids).
• **Regulation of blood pressure** by renin-angiotensin mechanism
• **Synthesis of erythropoietin**, a hormone which stimulates erythropoiesis
• **Production of vit. D_3 (active form of vit. D)** from vit. D_2, which stimulates absorption of calcium from gastrointestinal tract.

FACTORS AFFECTING RENAL FUNCTION

Kidney function is affected by following factors:
• Diffuse renal disease.
• **Pre-renal conditions—**Decreased renal blood flow as in dehydration, congestive cardiac failure and shock.
• **Post-renal conditions—**Obstruction to urinary outflow.

Box 2.1: Conditions with increased risk of chronic renal disease

- Diabetes mellitus
- Hypertension
- Autoimmune diseases like systemic lupus erythematosus
- Older age (GFR declines with age)
- Family history of renal disease
- Systemic infection
- Urinary tract infection
- Lower urinary tract obstruction

- **CHEMICAL EXAMINATION**

• Proteins • Urobilinogen
• Glucose • Blood
• Ketones • Hemoglobin
• Bilirubin • Myoglobin, Bile salts • Nitrite or leukocyte esterase

Proteins

Normally, kidneys excrete scant amount of protein in urine **(up to 150 mg/24 hours)**. These proteins include proteins from plasma (albumin) and proteins derived from urinary tract (Tamm-Horsfall protein, secretory IgA, and proteins from tubular epithelial cells, leucocytes, and other desquamated cells); this amount of proteinuria cannot be detected by routine tests. (Tamm-Horsfall protein is a **normal mucoprotein** secreted by ascending limb of the loop of Henle).

Proteinuria refers to protein excretion in urine greater than 150 mg/24 hours in adults.

- **Causes of Proteinuria** can be grouped as: 1**.** Glomerular proteinuria
  2. Tubular proteinuria
  3. Overflow proteinuria
  4. Hemodynamic (functional) proteinuria
  5. Post-renal proteinuria

1. Glomerular proteinuria: Proteinuria due to increased permeability of glomerular capillary wall is called as glomerular proteinuria.
There are two types of glomerular proteinuria: **selective** and **nonselective**. In early stages of glomerular disease, there is increased excretion of lower molecular weight selective proteins like **albumin and transferrin**.
With further glomerular damage, this selectivity is lost and larger molecular weight proteins (γ globulins) are also excreted along with albumin; this is called as **non-selective proteinuria**.

Selective and non-selective proteinuria can be distinguished by urine protein electrophoresis. In selective proteinuria, albumin and transferrin bands are seen, while in nonselective type, the pattern resembles that of serum (Fig. 1.3).
Serial estimations of urinary protein are also helpful inmonitoring response to treatment. Most severe degree of proteinuria occurs in **nephrotic syndrome**

Box 1.6: Nephrotic syndrome

- Massive proteinuria (>3.5 gm/24 hr)
- Hypoalbuminemia (<3.0 gm/dl)
- Generalised edema
- Hyperlipidemia (serum cholesterol >350 mg/dl)
- Lipiduria

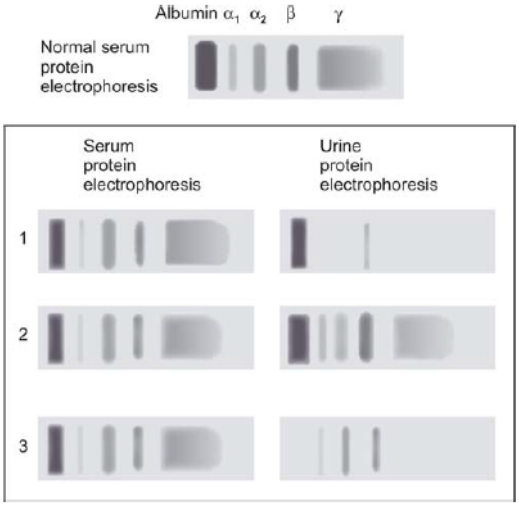

Fig. 1.3: Glomerular and tubular proteinuria. Upper figure shows normal serum protein electrophoresis pattern. Lower part shows comparison of serum and urine electrophoresis in (1) selective proteinuria, (2) non-selective proteinuria, and (3) tubular proteinuria

2. Tubular proteinuria proteinuria: Normally, glomerular membrane, although impermeable to high molecular weight proteins, allows ready passage to low molecular weight proteins like **β2-microglobulin**, **retinol-binding protein**, **lysozyme**, **α1-microglobulin**, and **free immunoglobulin light chains**. These low molecular weight proteins are actively reabsorbed by **proximal renal tubules**. In diseases such as **chronic pyelonephritis**, **heavy metal poisoning, tuberculosis of kidney, interstitial nephritis, cystinosis**, and **rejection of kidney transplant** involving mainly tubules, **these proteins are excreted in urine while albumin excretion is minimal.**
Urine electrophoresis shows prominent α- and β-bands (where low molecular weight proteins migrate) and a faint albumin band (Fig. 1.3). or using urine strip

3. Overflow proteinuria: When concentration of a low molecular weight protein rises in plasma, it "overflows" from plasma into the urine. Such proteins are **immunoglobulin light chains or Bence Jones proteins** (plasma cell dyscrasias), hemoglobin (intravascular hemolysis), myoglobin (skeletal muscle trauma), and lysozyme (**acute myeloid leukemia type M4 or M5**).

4. Hemodynamic proteinuria: Alteration of blood flow through the glomeruli causes increased filtration of proteins. Protein excretion, however, is transient. It is seen in **high fever**, **hypertension**, **heavy exercise**, **congestive cardiac failure, seizures, and exposure to cold**.

5. Post-renal proteinuria: This is caused by inflammatory or neoplastic conditions in **renal pelvis, ureter, bladder, prostate, or urethra.**

Table 1.4: Comparison of two tests for proteinuria

Parameter	*Reagent strip test*	*Sulphosalicylic acid test*
1. Principle	Colorimetric	Acid precipitation
2. Proteins detected	Albumin	All (albumin, Bence Jones proteins, hemoglobin, myoglobin)
3. Sensitivity	5-10 mg/dl	20 mg/dl
4. Indicator	Color change	Turbidity
5. Type of test	Screening	Confirmatory

Bence Jones Proteinuria

Bence Jones proteins are monoclonal immunoglobulin light chains (either k or γ) that are synthesized by **neoplastic plasma cells**. Excess production of these light chains occurs in plasma cell dyscrasias like multiple **myeloma and primary amyloidosis**. Because of their low molecular weight and high concentration, they are excreted in urine (overflow proteinuria). Bence Jones proteins have a characteristic thermal behaviour. When heated, Bence Jones proteins precipitate at temperatures between **40°C to 60°C** (other proteins precipitate between 60-70°C), and precipitate disappears on further **heating at 85-100°C** (while precipitate of other proteins does not). When cooled **(60-85°C),** there is **reappearance of precipitate of Bence Jones proteins**. This test, however, is not specific for Bence Jones proteins and both false-positive and -negative results can occur. This test has been replaced by protein **electrophoresis of concentrated urine sample** (Fig. 1.7).

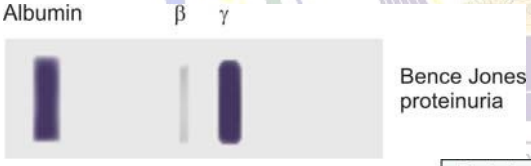

Fig. 1.7: Urine protein electrophoresis showing heavy Bence Jones proteinuria (red arrow) along with loss of albumin and other low molecular weight proteins in urine

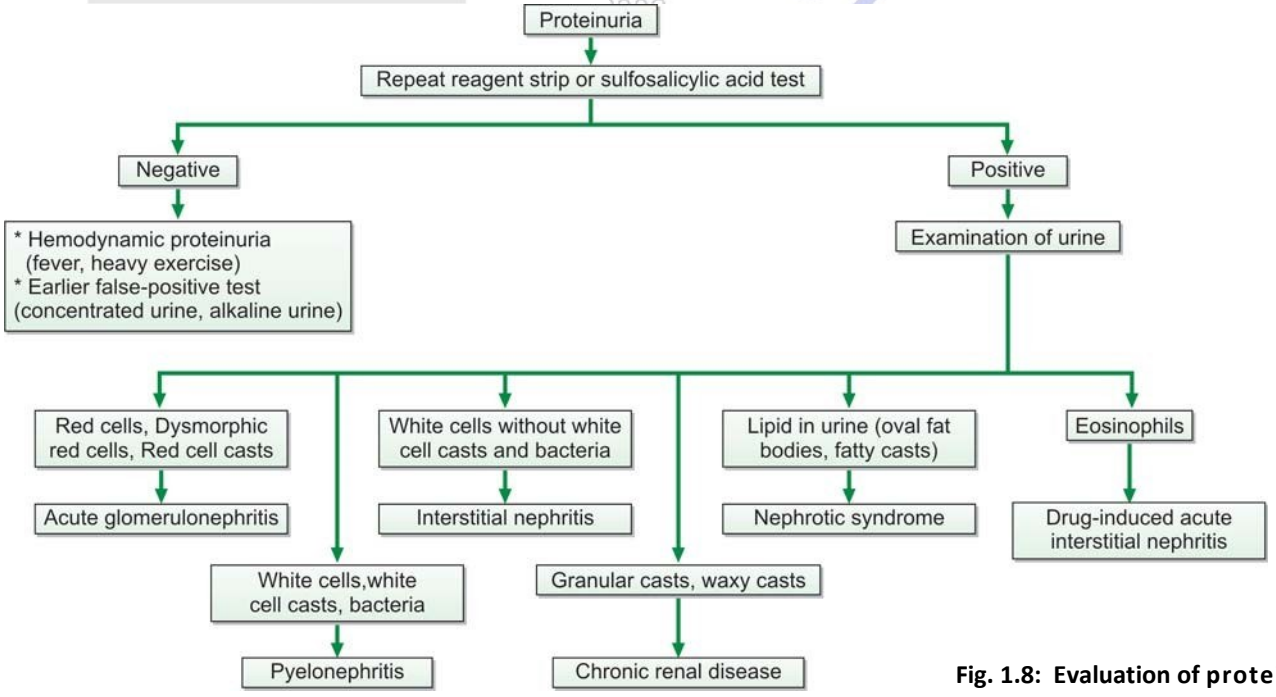

Fig. 1.8: Evaluation of proteinuria

Glucose

The main indication for testing for glucose in urine is detection of unsuspected **diabetes mellitus** or follow-up of known diabetic patients. Practically all of the glucose filtered by the glomeruli is reabsorbed by the proximal renal tubules and returned to circulation at concentration of 180 mg/dl. Normally a very small amount of glucose is excreted in urine (< 500 mg/24 hours or <15 mg/dl) that cannot be detected by the routine tests. **Presence of detectable amounts of glucose in urine is called as glucosuria or glycosuria**. Glycosuria results if the filtered glucose load exceeds the capacity of renal tubular reabsorption. Most common cause is hyper-glycemia from diabetes mellitus.

Causes of Glycosuria

1. **Glycosuria with hyperglycemia:**

- Endocrine diseases: diabetes mellitus, acromegaly, Cushing's syndrome, hyperthyroidism, pancrea- tic disease
- Non-endocrine diseases: central nervous system diseases, liver disorders
- Drugs: adrenocorticotrophic hormone, cortico- steroids, thiazides
- Alimentary glycosuria (Lag-storage glycosuria): After a meal, there is rapid intestinal absorption of glucose leading to transient elevation of blood glucose above renal threshold. This can occur in persons with gastrectomy or gastrojejunostomy and in hyperthyroidism. Glucose tolerance test reveals a peak at 1 hour above renal threshold (which causes glycosuria); the fasting and 2-hour glucose values are normal.

2. **Glycosuria without hyperglycemia**

- Renal glycosuria: This accounts for 5% of cases of glycosuria in general population. Renal threshold

Tests for Detection of Glucose in Urine

1. Copper reduction methods

Benedict's qualitative test: When urine is boiled in Benedict's qualitative solution, blue alkaline copper sulphate is reduced to red-brown cuprous oxide if a reducing agent is present (Fig. 1.9). The extent of reduction depends on the concentration of the **reducing substance**. This test, however, is not specific for glucose. Other carbohydrates (like **lactose, fructose, galactose, pentoses**), certain metabolites (**glucuronic acid, homogentisic acid, uric acid, creatinine), and drugs (ascorbic acid, salicylates, cephalosporins, penicillins, streptomycin, isoniazid, paraaminosalicylic acid, nalidixic acid, etc**.) also reduce **alkaline copper sulphate solution**.

$$\underset{\text{(Copper sulphate)}}{\text{Cupric ions (blue)}} + \text{Sugar} \xrightarrow[\text{Heat}]{\text{Alkali}} \text{Cuprous oxide (red)} + \text{Cuprous hydroxide (yellow)}$$

Fig. 1.9: Principle of Benedict's qualitative test for sugar in urine. Sensitivity is 200 mg of glucose/dl

2. Reagent strip method

This test is specific for glucose and is therefore preferred over Benedict's and Clinitest methods. It is based on **glucose oxidase-peroxidase reaction**. Reagent area of the strips is impregnated with two enzymes (**glucose oxidase and peroxidase) and a chromogen**. Glucose is oxidized by glucose oxidase with the resultant formation of hydrogen peroxide and gluconic acid.

Ketones

Excretion of ketone bodies (acetoacetic acid, β-hydroxy- butyric acid, and acetone) in urine is called as ketonuria. Ketones are breakdown products of fatty acids and their presence in urine is indicative of excessive fatty acid metabolism to provide energy.

Causes of Ketonuria

Normally ketone bodies are not detectable in the urine of healthy persons. If energy requirements **cannot be met by metabolism of glucose** (due to **defective carbohydrate metabolism**, **low carbohydrate intake**, or increased metabolic needs), then energy is derived from break- down of fats. This leads to the formation of ketone bodies his leads to the formation of ketone bodies (Fig. 1.12).

1. ***Decreased utilization of carbohydrates***

a. Uncontrolled diabetes mellitus with ketoacidosis: In diabetes, because of poor glucose utilization, there is compensatory increased lipolysis. This causes increase in the level of free fatty acids in plasma. Degradation of free fatty acids in the liver leads to the formation of acetoacetyl CoA which then forms ketone bodies.

b. Glycogen storage disease (von Gierke's disease)

2. ***Decreased availability of carbohydrates in the diet:***

A. Starvation
B. Persistent vomiting in children
C. Weight reduction program (severe carbohydrate restriction with normal fat intake)

3. ***Increased metabolic needs:***

A. Fever in children
B. Severe hyperthyroidism
C. Pregnancy
D. Protein calorie malnutrition

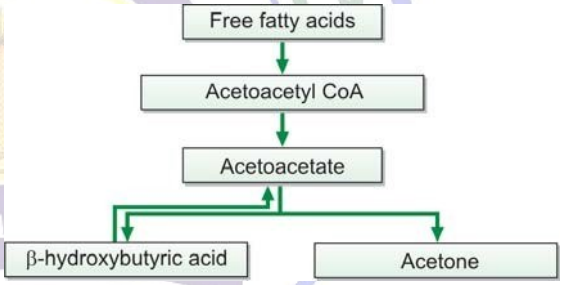

Fig. 1.12: Formation of ketone bodies. A small part of acetoacetate is spontaneously and irreversibly converted to acetone. Most is converted reversibly to β-hydroxybutyrate

Bile Pigment (Bilirubin)

Bilirubin (a breakdown product of hemoglobin) is undetectable in the urine of normal persons. Presence of bilirubin in urine is called as bilirubinuria.

There are two forms of bilirubin: **conjugated and unconjugated**. After its formation from hemoglobin in **reticuloendothelial system**, bilirubin circulates in blood bound to albumin. This is called as **unconjugated bilirubin**. Unconjugated bilirubin **is not water-soluble**, is bound to albumin, and cannot pass through the glomeruli; therefore, it does not appear in urine. The liver takes up **unconjugated bilirubin** where it combines with **glucuronic acid to form bilirubin diglucuronide** (conjugated bilirubiun). Conjugated bilirubin is water- soluble, is filtered by the glomeruli, and therefore appears in urine. **Detection of bilirubin in urine (along with urobili-nogen) is helpful in the differential diagnosis of jaundice (Table 1.6).**

Table 1.6: Urine bilirubin and urobilinogen in jaundice

Urine test	*Hemolytic jaundice*	*Hepatocellular jaundice*	*Obstructive jaundice*
1. Bilirubin	Absent	Present	Present
2. Urobilinogen	Increased	Increased	Absent

In acute viral hepatitis, bilirubin appears in urine even before jaundice is clinically apparent. In a fever of unknown origin bilirubinuria suggests hepatitis.

Presence of bilirubin in urine indicates **conjugated hyperbilirubinemia** (obstructive or hepatocellular jaundice). This is because only conjugated bilirubin is water-soluble. Bilirubin in urine is absent in hemolytic jaundice; this is because unconjugated bilirubin is water-insoluble.

Tests for Detection of Bilirubin in Urine

Bilirubin is converted to **non-reactive biliverdin on exposure to light** (daylight or fluorescent light) and on standing at room temperature. **Biliverdin cannot be detected by tests that detect bilirubin.** Therefore, fresh sample that is kept protected from light is required.

Methods for detection of bilirubin in urine are **foam test, Gmelin's test, Lugol iodine test, Fouchet's test**, **Ictotest tablet test, and reagent strip test**.

1. **Lugol iodine test**: Take 4 ml of Lugol iodine solution (Iodine 1 gm, potassium iodide 2 gm, and distilled water to make 100 ml) in a test tube and add 4 drops of urine. Mix by shaking. Development of **green color** indicates positive test.
2. **Reagent strips or tablets impregnated with diazo reagent**: These tests are based on reaction of **bilirubin with diazo reagent**; **color change** is proportional to the concentration of bilirubin. Tablets (Ictotest) detect 0.05-0.1 mg of bilirubin/dl of urine; reagent strip tests are less sensitive (0.5 mg/dl).

Bile Salts

Bile salts are salts of four different types of bile acids: **cholic, deoxycholic, chenodeoxycholic, and lithocholic.** These bile acids combine with **glycine or taurine** to form complex **salts or acids**. Bile salts enter the small intestine through the bile and act as detergents to emulsify fat and reduce the surface tension on fat droplets so that enzymes (lipases) can breakdown the fat. In **the terminal ileum**, bile salts are absorbed and enter in the blood stream from where they are taken up by the liver and re-excreted in bile (enterohepatic circulation).

Bile salts along with bilirubin can be detected in urine in cases of **obstructive jaundice.** In obstructive jaundice, bile salts and conjugated bilirubin regurgitate into blood from **biliary canaliculi** (due to increased intrabiliary pressure) and **are excreted in urine.** The test used for their detection is **Hay's surface tension test**. The property of bile salts to lower the surface tension is utilized in this test.

Take some fresh urine in a conical glass tube. Urine should be at the room temperature. Sprinkle on the surface particles of sulphur. If **bile salts are present**, **sulphur particles sink to the bottom because of lowering of surface tension by bile salts.** If sulphur particles remain on the surface of urine, **bile salts are absent**. **Thymol (used as a preservative) gives false positive test.**

Urobilinogen

Conjugated bilirubin excreted into the duodenum through bile is converted by bacterial action to urobilinogen in the intestine. Major part is eliminated in the feces. A portion of urobilinogen is absorbed in blood, which undergoes recycling (enterohepatic circulation); a small amount, which is not taken up by the liver, is excreted in urine. Urobilinogen **is colorless; upon oxidation it is converted to urobilin**, which is **orange-yellow in color**. Normally about 0.5-4 mg of urobilinogen is excreted in urine in 24 hours. Therefore, a small amount of urobilinogen is normally detectable in urine.

Causes of Increased Urobilinogen in Urine

1. **Hemolysis:** Excessive destruction of red cells leads to hyperbilirubinemia and therefore increased formation of **urobilinogen in the gut**. Bilirubin, being of unconjugated type, does not appear in urine. Increased urobilinogen in urine without bilirubin is typical of **hemolytic anemia**. This also occurs in **megaloblastic anemia** due **to premature destruction of erythroid precursors in bone marrow** (ineffective erythropoiesis).
2. **Hemorrhage in tissues:** There is increased formation of bilirubin from destruction of red cells.

Causes of Reduced Urobilinogen in Urine

1. **Obstructive jaundice:** In biliary tract obstruction, delivery of bilirubin to the intestine is restricted and very little or **no urobilinogen is formed.** This causes stools to become **clay-colored.**
2. **Reduction of intestinal bacterial flora:** This prevents conversion of bilirubin to urobilinogen in the intestine. It is observed in **neonates and following antibiotic treatment.**

Tests for Detection of Urobilinogen in Urine

Fresh urine sample should be used because on standing **urobilinogen is converted to urobilin**, which cannot be detected by routine tests. A timed (2-hour postprandial) sample can also be used for testing **urobilinogen**. Methods for detection of increased amounts of urobilinogen in urine are **Ehrlich's aldehyde test** and reagent strip test.

1. **Ehrlich's aldehyde test:** Ehrlich's reagent (*p*- dimethylaminobenzaldehyde) reacts with urobilinogen in urine to produce a **pink color**.

False-negative reaction can occur in the presence of **urinary tract infection** (nitrites oxidize urobilinogen to urobilin), and (ii) **antibiotic therapy** (gut bacteria which produce urobilinogen are destroyed).

2. **Reagent strip method:** This method is specific for urobilinogen. Test area is impregnated with either *p*-dimethylaminobenzaldehyde or 4-methoxy- benzene diazonium tetrafluoroborate.

Chemical Tests for Significant Bacteriuria (Indirect Tests for Urinary Tract Infection)

In addition to direct microscopic examination of urine sample, chemical tests are commercially available in a reagent strip format that can detect significant bacteriuria: **nitrite test and leucocyte esterase test**. These tests are helpful at places where urine microscopy is not available. If these tests are positive, urine culture is indicated.

1. **Nitrite test**: Nitrites are not present in normal urine; ingested nitrites are converted to **nitrate and excreted in urine**. If gram-negative bacteria (e.g. *E.coli, Salmonella, Proteus, Klebsiella*, etc.) are present in urine, they will reduce the **nitrates to nitrites through the action of bacterial enzyme nitrate reductase.** Nitrites are then detected in urine by reagent strip tests. As ***E. coli* is the commonest** organism **causing urinary tract infection**, this test is helpful as a **screening test for urinary tract infection.**

Some organisms like ***Staphylococci* or *Pseudomonas* do not reduce nitrate to nitrite and therefore in such infections nitrite test is negative**. Also, urine must be retained in the bladder for minimum of **4 hours for conversion of nitrate to nitrite** to occur; therefore, fresh early morning specimen is preferred. Sufficient dietary intake of nitrate is necessary. Therefore, **a negative nitrite test does not necessarily indicate absence of urinary tract infection**. The test detects about 70% cases of urinary tract infections.

2. **Leucocyte esterase test**: It detects **esterase enzyme released in urine from granules of leucocytes**. Thus the test is positive in **pyuria**. If this test is positive, urine culture should be done. The test is not sensitive to leucocytes < 5/HPF.

Blood Biochemistry test

Two biochemical parameters are commonly used to assess **renal function**: **blood urea nitrogen (BUN) and serum creatinine**. Although convenient, they are insensitive markers of glomerular function.

Blood Urea Nitrogen (BUN)

Urea is produced in the liver from amino acids (ingested or tissue-derived). Amino acids are utilized to produce energy, synthesize proteins, and are catabolized to ammonia. **Urea is produced in the liver from ammonia in the Krebs urea cycle**. **Ammonia is toxic and hence is converted to urea, which is then excreted in urine**.

Urea is completely filtered by the glomeruli, and about **30-40%** of the filtered amount is reabsorbed in the renal tubules depending on the person's state of hydration Blood level of urea is affected by a number of non- renal factors (e.g. **high protein diet, upper gastrointestinal hemorrhage, liver function, etc**.) and therefore utility of BUN as an indicator of **renal function is limited.** Also considerable **destruction of renal parenchyma** is required before elevation of blood urea can occur.

The term **azotemia refers to the increase in the blood level of urea**; uremia is the clinical syndrome resulting from this increase. If renal function is absent, BUN rises by 10-20 mg/dl/day.

Methods for estimation of BUN:

1. ***Diacetyl monoxime urea method***: This is a direct method. Urea reacts with diacetyl monoxime at high temperature in the presence of a strong acid and an oxidizing agent. Reaction of urea and diacetyl monoxime produces a yellow diazine derivative. The intensity of color is measured in a colorimeter or spectrophotometer.
2. ***Urease- Berthelot reaction***: This is an indirect method. Enzyme urease splits off ammonia from the urea molecule at 37°C.

Serum Creatinine

Creatinine is a nitrogenous waste product formed in muscle from creatine phosphate**.** Endogenous production of creatinine is proportional to muscle mass and body weight. Exogenous creatinine (from ingestion of meat) has little effect on daily creatinine excretion.

Serum creatinine is a more specific and more sensitive indicator of renal function as compared to BUN because: It is produced from muscles at a constant rate and its level in blood is not affected by diet, protein catabolism, or other exogenous factors; It is not reabsorbed, and very little is secreted by tubules.
However, because of significant kidney reserve, increase of serum creatinine level (**from 1.0 mg/dl to 2.0 mg/dl**) in blood does not occur until **about 50% of kidney function is lost.** Therefore, serum creatinine is not a sensitive indicator of **early renal impairment**. Adult males: 0.7-1.3 mg/dl. Adult females: 0.6-1.1 mg/dl.

Methods for Estimation of Serum Creatinine

The test for serum creatinine is cheap, readily available, and simple to perform. There are two methods that are commonly used:

1. Jaffe's reaction (Alkaline picrate reaction):
This is the most widely used method. Creatinine reacts with **picrate** in an alkaline solution to produce a **yellow-red color.** The color is measured in a spectrophotometer at 485 nm. Certain substances in plasma (such as glucose, protein, fructose, ascorbic acid, acetoacetate, acetone, and cephalosporins) react with picrate in a similar manner; these are called as **non-creatinine chromogens**.

2. Enzymatic methods: These methods use enzymes that cleave creatinine; hydrogen peroxide produced then reacts with phenol and a dye to produce a colored product, which is measured in a spectro- photometer.

Causes of Increased BUN/Creatinine Ratio (>20:1):

- Increased BUN with normal serum creatinine:
- Pre-renal azotemia (reduced renal perfusion)
- High protein diet
- Increased protein catabolism
- Gastrointestinal hemorrhage

Causes of Decreased BUN/Creatinine Ratio (<10:1)

- Acute tubular necrosis
- Low protein diet, starvation
- Severe liver disease
- Pregnancy
- Increasing age (reduction in muscle mass)